A test of the feasibility of using glue traps to sample the invertebrate fauna in bilby *Macrotis lagotis* foraging pits

Tamra F. Chapman

Department of Parks and Wildlife, Locked Bag 104, Bentley Delivery Centre, Bentley WA 6983 Email: tamra.chapman@dpaw.wa.gov.au

ABSTRACT

Interactions between mammals and invertebrates remain a gap in our knowledge of the role of fossorial foragers in Australian ecosystems. This is probably because digging mammals disappeared from the majority of their former range before they could be studied and because of the difficulties associated with sampling invertebrates in foraging pits. Bilbies were reintroduced into a rangeland restoration program in Western Australia, providing an opportunity to compare invertebrates in their foraging pits with the undisturbed surface soil. The challenge was to develop a method for collecting invertebrates while minimising disturbance of the floor of the foraging pits. This study investigated the feasibility of using folding cardboard glue traps to sample the invertebrates. The traps proved to be easy to deploy and collect and concerns that the traps on the soil surface would collect more soil than those in the foraging pits, thereby biasing the invertebrate sample, were not realised. No firm conclusions can be made about the interactions between bilby foraging and invertebrates from this short term study. However, there were indications that the abundance and composition of ant fauna may differ between foraging pits and the soil surface. Calculations were made to determine how many traps would have to be deployed to collect the representative sample needed to make valid statistical comparisons.

Key words: bilby, fossorial foragers, invertebrates, foraging pits, glue traps

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Introduction

The bilby *Macrotis lagotis* is a prolific digger (James and Eldridge 2007; Newell 2008) and invertebrates make up a large part of its diet (Gibson 2001). It is therefore likely that bilbies would have influenced invertebrate populations and assemblages via soil disturbance, seed dispersal, nutrient cycling, predation and competition for food (Gibb 2012; Silvey *et al.* 2015). However, the influence of bilby foraging on invertebrates is not well understood, probably because it disappeared from around 80% of its former range (Southgate 1990) before it could be studied, and because of the difficulties associated with sampling invertebrates in foraging pits.

Reintroduction of bilbies to habitats where they formerly occurred provides the opportunity to: study their interactions with invertebrate fauna; to establish their role in ecosystem function; and to measure their contribution to ecosystem restoration. Matuwa (Lorna Glen) is a former rangeland station, situated 1,100 km north east of Perth in Western Australia's Goldfields. It is jointly managed by the indigenous Martu people and the Department of Parks and Wildlife. Bilbies were re-introduced in 2007 (Morris and Dunlop 2008) to areas where predators are controlled via ongoing aerial baiting (Algar *et al.* 2013). This provided the means to compare invertebrate fauna in bilby foraging pits with that of undisturbed soil, but the challenge was to develop a method for collecting invertebrates, while minimising disturbance of the floor of the foraging pits.

There are many methods for sampling terrestrial invertebrates and each has its applications, advantages and limitations (Yi *et al.* 2012). Wet pitfall traps, 50 mm in diameter and 80 mm deep, containing ethylene glycol have been used to trap invertebrates on burrowing bettong *Bettongia lesueur* warrens that are also used by bilbies (Read *et al.* 2008). However, wet pitfall traps would not be suitable in foraging pits, because the soil in the floor of the pit would have to be excavated, prior to inserting the trap into the soil, to ensure it is flush with the soil surface. This would excavate the invertebrates present in the foraging pit before installation of the trap. A soil core sampler has been used to collect 70 mm diameter and 100 mm deep soil cores to study the invertebrate fauna of short-beaked echidna *Tachyglossus aculeatus* foraging pits (Eldridge and Mensinga 2007). The disadvantage of this method, however, is that it tends to record subterranean fauna and not mobile soil surface fauna and records a 'snapshot' of the fauna present, not the cumulative fauna captured over time like wet pitfall traps. Dry pitfall traps (4.4 L buckets) and hand searching have been used to study invertebrates in bettong / bilby habitats, but these methods are restricted to catching large invertebrates like spiders and scorpions (Silvey *et al.* 2015) and are not suitable for studying species assemblages.

Sticky traps are cardboard traps that have a coating of gel adhesive (usually polyisobutylene) on the floor and are typically used in commercial applications to monitor crawling insects like cockroaches. The goal of this study was to determine if glue traps could be used to compare the invertebrate fauna in bilby foraging pits with those on the undisturbed soil surface. The aim was to capture invertebrates that were mobile on the soil surface and thus

would represent the fauna targeted by bilby digging and / or the fauna that moved into foraging pits after digging by bilbies. The questions addressed in this study were: 1). Will the traps on the soil surface collect so much wind borne soil that they are ineffective for capturing invertebrates? 2). Are the invertebrates captured in bilby foraging pits different from those captured on the soil surface? 3). Is it feasible to use sticky traps to study the invertebrate fauna in bilby foraging pits? and if so, 4). How many traps would have to be deployed to collect the representative sample needed to make valid statistical comparisons?

Methods

The sticky traps used in this study were lo-line AgriSense™ 7 cm x 7cm folding cardboard cockroach traps. This size was chosen to fit into the foraging pits, which average around 1.4 L in volume (n = 29) at the study site (author's unpublished data), and to minimise the chances of capturing non-target vertebrate fauna such as small lizards and mammals. No attractant was used.

The traps were labelled using a felt tip permanent marker with a letter and number indicating the treatment and replicate. Twenty traps were placed into fresh (up to 5 days old) foraging pits and 20 were placed flat on the non-pit soil surface, approximately 1 m from each foraging pit (Figure 1. Example of a pair of traps installed in a bilby foraging pit and on the undisturbed soil surface.).

Traps on the soil surface were fixed in position using a tent peg to prevent movement by wind or fauna. The traps were installed on the morning of 13 April 2012 and collected on the morning of 16 April 2012, so that they had been in place for three days and three nights. Upon collection, the traps were placed into a cardboard box and fumigated with domestic insecticide, to ensure that all the invertebrates had been euthanized. Freezing has since been proposed as a more suitable means of euthanizing and preserving the trapped specimens (Brian Heterick pers. comm.).

Image analysis was used to determine if the amount of soil captured on the traps varied significantly between foraging pits and undisturbed soil. Each trap was photographed from directly overhead, the photographs were imported into the digital image analysis software ImageJ 1.45m (Abràmoff *et al*., 2004) and the coverage of soil was determined via binary masking and particle analysis in accordance with the operation manual (Ferreira and Rasband, 2010-2011). The images were scaled in proportion to the known width of the traps and colour thresholding was used to isolate the soil from the invertebrates and background of the traps. The images were then converted to 16-bit format and the particle analysis menu was used to determine the area and proportion of the trap covered in soil. The data were log normal transformed to ensure they met the assumptions of the test and compared using one-way ANOVA via JMP 9 software (SAS Institute Inc.).

Figure 1. Example of a pair of traps installed in a bilby foraging pit and on the undisturbed soil surface.

The glue covered region of base of the trap was cut away with scissors and immersed in De-Solve-It® orange oil based solvent in a Petri dish for around 12 hours. Invertebrate specimens were retrieved from the dish using forceps and / or a paint brush and transferred to a small glass vial, containing 70% ethanol, which was labelled with the trap details. If any specimens had residual glue on them, they were returned to a fresh solvent bath and monitored until the glue had completely dissolved.

Ants were identified to species and non-ants were identified to morphospecies within orders. Due to the inadequate capture of non-ants, the analyses were restricted to ant fauna only. EsimateS 9.1.0 (Colwell 2013) software was used to calculate species richness, abundance, individual rarefaction curves and Shannon diversity index (H). $H = \text{sum}((n_i/n)^2)\ \ln(n_i/n))$, where n_i = the number of individuals of taxon i, and is a measure of evenness of species among the individuals recorded (Colwell 2013). It ranges from 0 to 1 and higher values represent many taxa each with a small number of individuals.

Matched pair tests were used via JMP 9 software (SAS Institute Inc.) to compare species richness, abundance and Shannon diversity index between foraging pits and the soil surface. Data for abundance were log transformed to ensure they met the assumptions of the test and compared using a paired t-test. Wilcoxon Signed Rank tests were used for Shannon diversity index, because the data were in percentage form, and for richness, because the data did not meet the assumptions of a parametric test.

Similarity percentage analysis (after Clarke 1993) was used to identify which taxa were primarily responsible for the differences in ant fauna composition between foraging pits and the soil surface using PAST 3 software (Hammer 1999-2013). The sampling effort needed to detect 90%, 95% and 99% of the estimated total number of species present was calculated using the method of Chao *et al*. (2009) via the spreadsheet provided in the supplementary material.

Results

There was no difference in the cover of soil between traps in the foraging pits (61 ± 5%, mean ± s.e.) and those on the surface (63 ± 7%, P = 0.79).

Non-ants

The total number of morphospecies of non-ants was

similar for foraging pits and the soil surface, but the total number of individuals recorded was slightly higher for foraging pits (Table 1. Comparison of richness and abundance for non-ant invertebrate orders trapped in foraging pits (n = 20) and on the soil surface (n = 20).). The low number of detections meant that non-ant data could not be statistically tested, but of note was the higher richness and abundance of *Diptera* and *Hymenoptera* morphospecies in foraging pits than on the soil surface

Table 1. Comparison of richness and abundance for non-ant invertebrate orders trapped in foraging pits (n = 20) and on the soil surface (n = 20).

Treatment	Foraging pits		Soil surface	
Order	Taxa	Individuals	Taxa	Individuals
Acarina	2	3	2	2
Aranaeomorpha	2	2	4	4
Arthropleona	2	5	3	6
Blattodea	-	-	1	1
Coleoptera	1	1	-	-
Diptera	9	16	6	7
Hemiptera	2	2	3	3
Hymenoptera	14	16	9	10
Isoptera	1	1	-	-
Orthoptera	2	2	4	4
Siphonaptera	-	-	1	1
Symphypleona	1	6	1	1
Unknown	1	1	2	2
Total	37	55	36	41

(Table 1. Comparison of richness and abundance for non-ant invertebrate orders trapped in foraging pits (n = 20) and on the soil surface (n = 20).).

Ants

Ant species richness did not differ between foraging pits and the soil surface (S = -17.5, P = 0.4272), but the number of individuals was significantly lower (t = 1.53, P = 0.0494) and Shannon diversity index was significantly

Table 2. Comparison of the ant fauna trapped in bilby foraging pits (n = 20) and the soil surface (n = 20).

Species	Foraging pits	Soil surface
Aenictus turneri (Forel)	1	1
Doleromyrma rottnestensis (Wheeler)	-	1
Iridomyrmex agilis (Forel)	3	3
Iridomyrmex chasei (Forel)	318	1,170
Iridomyrmex dromus Clark	-	8
Iridomyrmex exsanguis (Forel)	1	3
Camponotus arenatus (Shattuck and McArthur)	-	1
Camponotus aurocinctus (F. Smith)	3	-
Camponotus claripes Mayr	-	2
Camponotus donnellani (Shattuck and McArthur)	1	-
Camponotus guidae (McArthur)	4	3
Camponotus longifacies (McArthur)	2	-
Melophorus bagoti (Lubbock)	2	2
Melophorus fieldi (Forel)	1	-
Melophorus ludius sulla (Forel)	8	-
Melophorus marius (Forel)	-	3
Melophorus turneri (Forel)	1	1
Melophorus sp. (JDM 783)	-	1
Melophorus sp.	-	1
Opisthopsis haddoni rufoniger (Forel)	-	1
Opisthopsis sp.	-	1
Meranoplus sp. (JDM 74)	1	-
Monomorium fieldi (Forel)	3	3
Monomorium nanum (Heterick)	1	-
Monomorium rothsteini (Forel)	3	-
Pheidole sp. near variabilis (JDM 177)	13	9
Solenopsis clarki Crawley	1	-
Tetramorium megalops Bolton	8	5
Anochetus rectangularis Mayr	-	1
Taxa	**19**	**20**
Individuals	**375**	**1,220**
Shannon diversity index	**0.81**	**0.28**

higher (S = 105.00, P < 0.0001) for foraging pits (Table 2. Comparison of the ant fauna trapped in bilby foraging pits (n = 20) and the soil surface (n = 20).). Ten species were exclusively recorded in foraging pits and nine species were exclusively recorded on the soil surface (Table 2. Comparison of the ant fauna trapped in bilby foraging pits (n = 20) and the soil surface (n = 20).).

The largest contributor to dissimilarity in the ant fauna was by *Iridomyrmex chasei* (Forel), which was more abundant on the soil surface than in foraging pits (Table 3. The top 90% of contributors to dissimilarity in ant fauna between foraging pits and the soil surface.). *Pheidole* sp. near *variabilis*, *Tetramorium megalops*, *Melophorus ludius sulla* and *Camponotus guidae* were more abundant in foraging pits, but the magnitude of the difference was relatively small for these species (Table 3. The top 90% of contributors to dissimilarity in ant fauna between foraging pits and the soil surface.).

The shape of the rarefaction curves indicated that ants were under-sampled for both foraging pits and the soil surface, since richness showed no tendency to plateau for the total number of individuals collected (Figure 2. Comparison of individual rarefaction curves for ants trapped in a). foraging pits and b). on the soil surface shown with 95% confidence intervals.).

Modelling showed that to represent 95% of the total ant fauna estimated to be present, the number of

Table 3. The top 90% of contributors to dissimilarity in ant fauna between foraging pits and the soil surface.

Taxon	Mean dissimilarity	Contribution (%)		Mean abundance per trap	
		Taxa	Cumulative	Foraging pits	Soil surface
Iridomyrmex chasei (Forel)	54.2	80.9	80.9	15.9	58.5
Pheidole sp. near *variabilis* (JDM 177)	2.5	3.7	84.6	0.7	0.5
Tetramorium megalops (Bolton)	1.6	2.4	87.0	0.4	0.3
Melophorus ludius sulla (Forel)	1.2	1.9	88.9	0.4	-

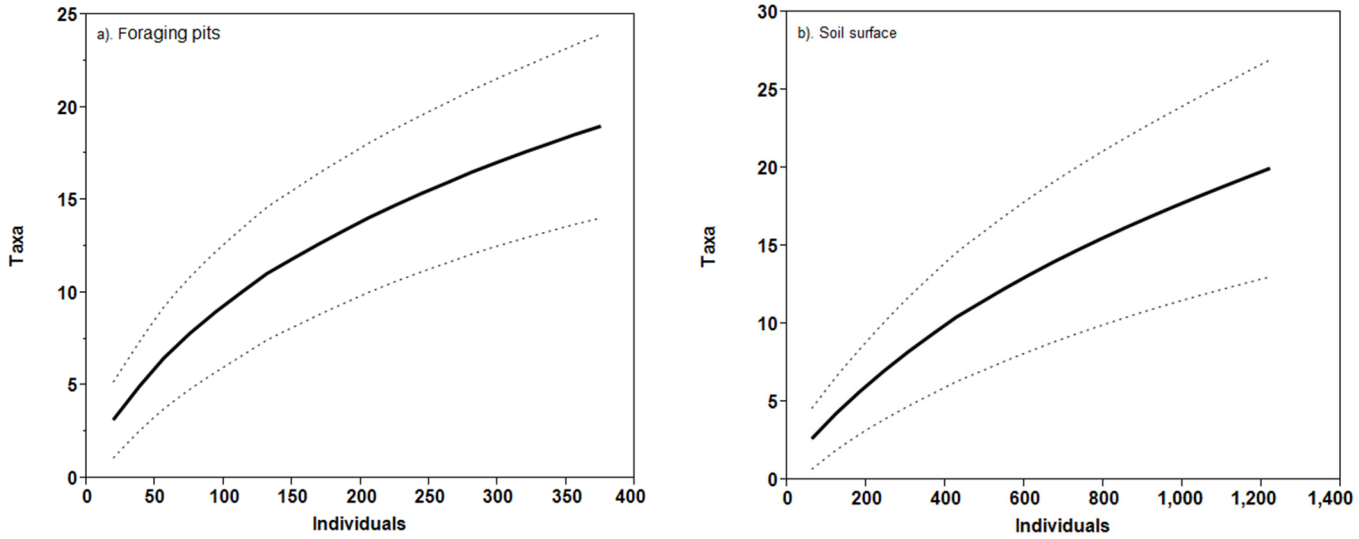

Figure 2. Comparison of individual rarefaction curves for ants trapped in a). foraging pits and b). on the soil surface shown with 95% confidence intervals.

Table 4. Data used to estimate sampling effort for abundance, where n = number of individuals, S_{obs} = observed species richness, S_{est} = estimated species richness, based on the Chao 1 estimator, f_1 = number of singletons, f_2 = number of doubletons, q_0 = probability that the next individual sampled represents a previously undetected species and g represents the number of additional individuals needed to detect 90%, 95% and 99% of S_{est}. For the algorithms see Chao et al. (2009).

Additional individuals required	n	S_{obs}	S_{est}	f_1	f_2	q_0	g = 0.90	g = 0.95	g = 0.98
Foraging pits	375	19	35.0	8	2	0.0213	1,140	1,660	2,347
Soil surface	1,220	20	40.3	9	2	0.0074	4,435	6,338	8,853

Table 5. Data used to estimate sampling effort for traps, where t = number of traps set, T = number of incidences, S_{obs} = observed species richness, S_{est} = estimated species richness, based on the Chao 2 estimator, Q_1 = number of species represented in one trap, Q_2 = number of species represented in two traps, q_0 = probability that the next trap will contain a species new to the survey and g represents the number of additional traps needed to detect 90%, 95% and 99% of Sest. For the algorithms see Chao et al. (2009).

Additional traps required	t	T	S_{obs}	S_{est}	Q_1	Q_2	q_0	g = 0.90	g = 0.95	g = 0.99
Foraging pits	20	63	19	28.6	9	4	0.1429	26.5	41.7	76.9
Soil surface	20	53	20	54.2	12	2	0.2264	105.9	145.8	238.3

additional traps that would have to be deployed was 42 (1,660 individuals) for foraging pits and 146 (6,338 individuals) for the soil surface (see Table 4 & Table 5).

Discussion

The sticky traps used in this study proved to be easy to deploy and collect. Contrary to the prediction that traps on the soil surface would collect more soil than those in the foraging pits, thereby biasing the invertebrate sample, both sets of traps were around 60% covered with soil after three days. This suggests that the low profile and cover provided by the fold-over top may have prevented the traps from collecting excessive amounts of soil, despite exposure to wind on the soil surface.

Few conclusions can be drawn about non-ant invertebrate fauna due to the small number of taxa and individuals captured. Of interest, however, was the slightly higher diversity and abundance of diptera (flies) and hymenoptera (wasps) captured in foraging pits. Since these are diurnal flying insects, they would not be regarded as prey species of the nocturnal fossorial bilby (Gibson 2001). Therefore, they may be attracted to some other element of the environmental conditions related

to foraging pits. For instance, they may be associated with the grass hummocks in proximity to the foraging pits or the more moderate microclimate conditions in the foraging pits (Eldridge and James 2009). They may also prey on, or parasitise, the ants and their larvae that have been exposed in the pits by bilby digging.

Both foraging pits and the soil surface were dominated by *Iridomyrmex chasei*, but it was more prevalent and dominant on the surface. This is likely to be because *Iridomyrmex* is an aggressive and dominant genus, that excludes and preys on other fauna, and is more common in open habitats (Andersen 1997). One reason *I. chasei* was so dominant on the soil surface may be because the traps were held in place using a tent peg and the disturbance caused by the installation of the traps may have attracted individuals to these micro-sites. In contrast, foraging pit traps were simply placed into pits with minimal disturbance. Presumably, however, the digging of foraging pits by bilbies would also represent a disturbance of the kind likely to attract ants like *I. chasei*. An alternative means of securing surface traps would be to place a rock on the top of the traps, which would camouflage the traps, prevent movement by wind and minimise soil surface disturbance on installation.

This was a short term study, so no firm conclusions can be drawn about the interactions between bilby foraging and invertebrates. However, the results suggested that foraging pits and the soil surface may vary in invertebrate abundance and composition. In particular, the Shannon diversity index for foraging pits exceeded that of the soil surface and the species that were more common in foraging pits included *Pheidole sp.*, *Tetramorium* sp. and *Melophorus* sp. These are seed harvesting and caching species (Briese and Macauley 1981) and, therefore, it may be that bilbies feed on these non-aggressive ant species, their larvae and / or their seed caches (Gibson *et al.* 2002). Alternatively, these ants may, themselves, forage in the pits because they contain exposed seed caches and because the pits accumulate wind borne seed from the surrounding habitat matrix (Eldridge and James 2009; Newell 2008).

This study has shown that sticky traps are a feasible means of studying interactions between invertebrates and bilbies and, potentially therefore, the role of bilbies in rangeland restoration. It has also helped establish the minimum number of traps that would be needed to adequately represent 95% of the fauna present in such a study; 62 (2,035 individuals) for foraging pits and 166 (7,558) for the soil surface.

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